The Effects of 8 Weeks of Endurance Running on Hepcidin Concentrations, Inflammatory Parameters, and Iron Status in Female Runners

Irena Auersperger, Bojan Knap, Ales Jerin, Rok Blagus, Mitja Lainscak, Milan Skitek, and Branko Skof

Exercise-associated iron deficiency is a common disorder in endurance athletes. The authors investigated the effects of long-term endurance exercise on hepcidin concentrations, inflammatory parameters, and iron status in moderately trained female long-distance runners. Eighteen runners were assigned to either an interval- or a continuous-training exercise group. The physical training consisted of two 3-week progressive overload periods, each followed by a week’s recovery, and concluded with a 10- or 21-km competitive run. Samples were taken 6 times during the 8-wk training program, first at baseline (BPre), then after the first and second 3-wk training loads (TPost1, TPost2), after each recovery week (Recovery1 and Recovery2), and poststudy (BPost). Soluble transferrin receptor (sTfR) concentrations were increased in Recovery2 and BPost compared with BPre ($p = .02$), hemoglobin decreased in TPost1 and TPost2 ($p < .001$), and red blood cells decreased in TPost2 ($p = .01$). Hepcidin decreased with time in TPost1 and in BPost compared with BPre ($p < .001$) and increased in TPost2 compared with TPost1 ($p < .001$). No differences over time were found for high-sensitivity C-reactive protein. The main findings of the current study indicate that serum hepcidin and sTfR were affected after 8 weeks of endurance running in women. No positive relation was found with inflammation.

Keywords: iron metabolism, monitoring training intensity, inflammation, women athletes

Athletes, in particular women and adolescents, are at risk for depleting their iron stores. Untreated iron deficiency can, in more severe cases, result in iron-deficiency anemia (Beard & Tobin, 2000; Williams, 2005). Iron is an essential constituent of oxygen-transport proteins (e.g., hemoglobin and myoglobin) and serves as an important cofactor in mitochondrial energy production. Hence, inadequate iron stores may decrease physical performance (Anker et al., 2009; Schumacher, Schmid, König, & Berg, 2002; Williams, 2005). The mechanisms contributing to iron loss during exercise are hemolysis, hematuria, sweating, and gastrointestinal bleeding (Babic et al., 2001; DeRuisseau, Cheuvront, Haymes, & Sharp, 2002; McInnis, Newhouse, von Duvillard, & Thayer, 1998; Zoller & Vogel, 1994). In addition, recent reports suggest that inflammation can induce iron sequestration via hepcidin activation (Kemna, Pickkers, Nemeth, van der Hoeven, & Swinkels, 2005). Hepcidin blocks the release of iron from macrophages and inhibits absorption of iron from the gut (Nemeth, Rivera, et al., 2004, Nemeth, Tuttle, et al., 2004), ultimately causing functional or absolute iron deficiency. The mode, intensity, and duration of exercise can increase the concentrations of cytokines (Fischer, 2006; Ostrowski, Rohde, Zacho, Asp, & Pedersen, 1998). Interleukin 6 (IL-6), a pleiotropic inflammatory cytokine, is produced postexercise in greater amounts than any other cytokine, and one of its biological activities is the generation of acute-phase reactants such as C-reactive protein (CRP; Kishimoto, Akira, Narazaki, & Taga, 1995). IL-6 is also the predominant mediator of the iron-regulatory hormone hepcidin (Nemeth, Rivera, et al., 2004). An increase of inflammation-induced hepcidin may cause a rapid decrease in plasma iron concentrations, which will eventually decrease hemoglobin synthesis (Lainscak, von Haehling, & Anker, 2009; Nemeth, Tuttle, et al., 2004).

Few studies have examined the effects of exercise on hepcidin. Most of them have focused on urinary hepcidin (Roecker, Meier-Buttermilch, Brechtel, Nemeth, & Ganz, 2005; Peeling et al., 2009a,b,c). The general outcomes of the research have shown transient increases in urinary hepcidin concentrations after one or two bouts of exercise. On the other hand, Troadec et al. (2009) reported that submaximal concentric exercise did not modulate serum or urinary hepcidin concentrations. There is only one study that evaluated whether regular exercise could have the same effects. Karl et al. (2010) showed that serum hepcidin concentrations were not affected by 9 weeks of basic combat training for female soldiers but were associated with iron status and inflammation. This recent research suggests that inflammation in relation...
to the iron-regulatory hormone hepcidin might be an important mediator in exercise-associated iron deficiency in athletes.

We therefore evaluated the effects of 8 weeks of endurance exercises, controlled for training intensity, duration, and number of completed sessions, on serum hepcidin concentrations, inflammatory parameters, and iron status in moderately trained female long-distance runners.

**Methods**

**Study Design and Participants**

This was a prospective study in 18 moderately trained female runners. Participants entered the study after being informed about the purpose, potential benefits, and possible risks of the training program. Subjects were eligible for inclusion in the study if they met the following criteria: regular menstrual cycles (defined as 9–12 per year occurring at regular intervals), regular dietary intake of animal product (defined as intake of at least one serving of meat, fish, or poultry on most days), no use of iron supplements, and no regular use of medication. All women with anemia (hemoglobin [Hb] <120 g/L) or iron deficiency (ferritin concentrations <10 μg/L) were excluded from the study. According to detailed disease history, physical examination before the study begin, and baseline laboratory analysis, we can say they were free of any acute or chronic inflammatory condition. Mean leukocyte values over time were within the normal range and are presented in Table 3.

The runners were divided into two groups who were familiar with and able to complete the training program: interval training (n = 10) or continuous training (n = 8). The groups also differed in their performance characteristics (limit result for 2,400-m Cooper test <11 min for interval and ≥11 min for continuous; Table 1). The study protocol was approved by the National Ethics Committee of Slovenia, and all participants gave written informed consent.

**Experimental Overview**

The study was completed during the intensified training phase for the 10-km (n = 5) or the 21-km (n = 13) run competition at the International Ljubljana Marathon in October 2008. Before beginning the training phase, all runners completed a 2-week run-in period of low-intensity physical training to ensure familiarity with experimental procedures and to have reached a nonfatigued state. At the beginning of the experimental training period, which lasted for 8 weeks, all runners completed incremental tests to exhaustion, ran a 2,400-m time trial (Cooper test), and had their anthropometric status measured. The physical training program consisted of two 3-week progressive overload periods each followed by a week’s recovery. After the second recovery week, the runners participated in the race, and after a further week of recovery, we again measured their physiological status. Blood samples were taken at six time points: baseline-pre (BPre) before the training program, after completion of each 3-week training load (TPost1 and TPost2), after each recovery week (Recovery1 and Recovery2), and poststudy (BPost) 1 week postcompetition. The protocol is shown in Figure 1.
Experimental Procedures

Running Training. The participants in the interval group had four training sessions per week in training-load phases consisting of two interval trainings (one at 88–95% maximum heart rate [HR\text{max}], the second up to 100% HR\text{max}) and two easy runs (at 70–87% HR\text{max}) of 6–8 km and 12–18 km. The continuous group had three training sessions per week in training-load phases consisting of one easy interval training (fastleak, or speed play, at 80–90% HR\text{max}) and two easy runs (at 70–87% HR\text{max}) similar to those in the interval group. Based on the runners’ HR\text{max}, which we determined during the BPre incremental test to exhaustion, we set each runner’s training protocol to match the same training stimulus defined for the group. The runners completed a 2,400-m time trial (Cooper) on an outdoor 400-m Tartan track before commencing training and then every 14 days during the testing period. In both groups, recovery-period interval training was replaced with an easy run of 6–8 km. All training sessions were supervised by at least one qualified athlete coach and one member of the research group.

Anthropometry. Each runner’s mass, height, and body-mass index were measured BPre and BPost. Body-mass index was calculated as body weight (kg)/height (m²). Incremental Test to Exhaustion. All the women completed an incremental test to exhaustion on a treadmill at BPre and BPost. Each runner had previous experience with treadmill running and testing. After a 6-min warm-up, an incremental protocol on a calibrated treadmill (Technogym, UK) with a 2% incline was applied. The starting speed was 3 km/hr, with speed increments of 2 km/hr every 2 min. The runners walked the first stage and then ran until volitional exhaustion. The last half or full stage that a subject could sustain (for either 1 min or 2 min) was defined as that individual’s maximal speed. During recovery, the subjects walked at 5 km/hr for 5 min. Maximum oxygen consumption (VO\text{2max}) was assessed using a Cosmed K4b2 (Rome, Italy) spirometric system (McLaughlin, King, Howley, Bassett, & Ainsworth, 2001). Heart rate (HR) was recorded continuously during the test using telemetric heart monitors (Polar Electro, Oulu, Finland) and stored on a computer.

Monitoring Training Load. Each participant wore an HR monitor (interval group, Polar RS800sd; continuous group, Polar RS400sd; Finland) during the process to record training, not including warm-up and cooldown intervals but noting the recovery interval on training interval days. Weekly exercise scores were calculated from the training loads using a method based on the HR-zone training-point system (Edwards, 1996). The length of time (in minutes) in various HR-based zones was computed from the HR monitor, multiplied by the value of the zone, and summed to derive \( \sum \text{HR-zone training points} \).

Laboratory Tests. After an overnight fast, blood samples were taken between 7 and 8 a.m. to avoid variations in circadian rhythm. All participants were asked to refrain from drinking coffee, tea, chocolate, or cola drinks, and they were asked to avoid alcohol for the prior 24 hr. All blood samples were taken with the subject in a seated position. EDTA blood samples were taken for the analysis of leukocytes, red blood cells (RBC), Hb, and Hct in an Advia 2010 analyzer (Siemens Healthcare, Erlangen, Germany). To further investigate the effect of hemodilution on the measured variables, plasma, blood and RBC volume were estimated using the formulas of Dill and Costill (1974); initial BV (BV\text{i}) was set at 100, where BV is blood volume, PV is plasma volume, RCV is red-cell volume, suffix 1 is the variable before exercise (BPre), and suffix 2–6 is the variable during other times of measurement.

\[
\begin{align*}
BV_{2–6} &= BV_1 \times \frac{(Hb_2–6/Hb_1)}{100} \\
PV &= BV – RCV \\
RCV &= BV \times (Hct\%) \\
% \text{ change in } BV &= 100 \times (BV_{2–6} – BV_1) \\
% \text{ change in } RCV &= 100 \times (RCV_{2–6} – RCV_1) \\
% \text{ change in } PV &= 100 \times (PV_{2–6} – PV_1)
\end{align*}
\]

For analyses of serum iron (Fe), soluble transferrin receptor (sTfR), ferritin, CRP, IL-6, and hepcidin, blood samples were collected without additives; after centrifugation, the serum was stored at –20 °C. Fe was measured spectrophotometrically in an Advia 1800 analyzer (Siemens Healthcare, Erlangen, Germany), sTfR was measured using immunonephelometry on the BN system (Siemens Healthcare), and ferritin was measured by immununoturbidimetric assay in an Olympus AU400 analyzer (Beckman Coulter, Brea, CA). CRP was measured using a chemiluminescent immunometric high-sensitivity assay with a detection limit of 0.3 mg/L (Insmut, Siemens Healthcare, Erlangen, Germany), and IL-6 was measured by electrochemiluminescence assay with a detection limit of 2 ng/L (Cobas e411 analyzer, Roche Diagnostics, Mannheim, Germany). Hepcidin was measured by the ELISA method with an analytical sensitivity of 4 μg/L (IBL, Hamburg, Germany).

Data Analysis

Results are expressed as \( M (\pm SD) \). Differences between groups in baseline values of anthropometric measurements, VO\text{2max}, and results of Cooper tests were analyzed with \( t \) tests. A Welch correction was used to adjust for unequal variances in the groups. The effects of group on BPre and BPost times and Group × Time interaction on exercise intensity were analyzed with two-way repeated-measures ANOVA; this method was also used to analyze group, time, and Group × Time effects on laboratory markers. Analysis of contrasts was used to determine where there were specific, preplanned differences. The \( p \)
Results

Physical Responses to Training

All 18 runners completed the study. As shown in Table 1, there were no significant baseline differences between the two groups in age, height, mass, body-mass index, or VO2max, but the Cooper test showed differences (p < .001). At completion of the program, a significant improvement in physical performance was observed in both groups of runners. The increase in VO2max from BPre to BPost was +4.7% in the interval group and +2.21% in continuous, and the time needed to complete the Cooper test was shorter (interval –6.1%, continuous –5.5%). Running distance per week in the first training load increased in interval and continuous from 25.02 (± 12.49) to 41.60 (± 5.43) and from 23.19 (± 2.88) to 29.4 (± 8.94) km, respectively. In the second training load, interval and continuous completed 37.53 (± 9.34) up to 44.07 (± 8.13) and 21.47 (± 10.02) up to 29.24 (± 13.94) km/week, respectively. Mean km/week for interval and continuous were 17.07 (± 7.39) and 18.66 (± 6.93) in Recovery1 and 10.43 (± 5.34) and 8.84 (± 2.7) in Recovery2, respectively. The same trend in weekly training loads was observed when using a method based on the HR-zone training-point system. The total distance, training duration, and VO2max were significantly greater (+32.4%) in interval than in continuous (p = .0112). Exercise scores based on Σ HR-zone training points were 15.9% higher in interval but were not significant (p = .0644). All participants finished either the 21-km (interval = 7, continuous = 6) or 10-km (interval = 3, continuous = 2) run, improving their personal best. Mean results for interval and continuous for the 21-km run were 1:38:10 (± 8:13) and 1:59:10 (± 3:27) and for the 10-km run were 46:50 (± 1:27) and 58:07 (± 5:10), respectively.

Table 1 Baseline Characteristics of 18 Female Runners, M (SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, years</th>
<th>Mass, kg</th>
<th>Height, cm</th>
<th>Body-mass index</th>
<th>VO2max, ml · kg⁻¹ · min⁻¹</th>
<th>Cooper test, s/2,400 m</th>
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<td>Interval, n = 10</td>
<td>32.9 (5.7)</td>
<td>59.72 (6.69)</td>
<td>171.0 (6.6)</td>
<td>20.27 (1.87)</td>
<td>48.71 (6.81)</td>
<td>610.90 (37.44)</td>
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<tr>
<td>Continuous, n = 8</td>
<td>31.6 (4.8)</td>
<td>60.23 (9.17)</td>
<td>164.7 (4.8)</td>
<td>22.23 (3.53)</td>
<td>46.01 (4.11)</td>
<td>752.29 (57.37)*</td>
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</table>

*Significantly different from interval group (p < .05).
Table 3  Selected Laboratory Markers at Baseline (BPre), During the First and Second Training Loads (TPost1, TPost2), After Both Recovery Phases (Rec1, Rec2), and Poststudy (BPost) for the Interval- and Continuous-Training Groups, M (SD)

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<th>Measure</th>
<th>BPre</th>
<th>TPost1</th>
<th>Rec1</th>
<th>TPost2</th>
<th>Rec2</th>
<th>BPost</th>
<th>Group</th>
<th>Time</th>
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<td>(0.59)</td>
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*Note. hsCRP = high-sensitivity C-reactive protein; RBC = red blood cells; sTfR = soluble transferrin receptor.

*Significantly different from BPre (p < .05).
RBC

Results for RBC are also shown in Table 3. The effect of time was significant. At TPost1 and TPost2, the women had lower concentrations of RBC than at BPre, but this reached statistical significance only at TPost2 ($p = .01$). There were no significant effects of group or Group $\times$ Time ($p = .77$, $p = .82$).

sTfR

sTfR concentrations measured at BPre, Recovery2, and BPost are shown in Table 3. The effect of time was significant ($p = .02$), and sTfR concentrations were higher at Recovery2 and BPost than at BPre ($p = .02$, both cases).

Ferritin

Serum ferritin concentrations measured at BPre, Recovery2, and BPost are shown in Table 3. Compared with BPre, concentrations of ferritin at Recovery2 decreased nonsignificantly (time $p = .07$, group $p = .20$).

IL-6 and High-Sensitivity CRP

IL-6 concentrations at all time points were below the detectable plasma concentrations of 2 ng/L. No significant effect of time or group was found for high-sensitivity CRP (hsCRP) concentrations (time $p = .56$, group $p = .9$; Table 3).

Hepcidin

Hepcidin concentrations are shown in Figure 2. There was a significant time effect ($p < .001$) but no Group $\times$ Time or group effect ($p = .33$, $p = .22$). Participants in both groups had lower average values of hepcidin at TPost1 and BPost than at BPre ($p < .001$). At TPost2, concentrations of hepcidin were greater than those recorded at TPost1 ($p < .001$). There were no differences between hepcidin concentrations at Recovery1 and Recovery2 ($p = .85$).

Vascular Volumes

Changes in vascular volumes are shown in Table 4. No significant changes between interval and continuous groups were found for vascular volumes ($p > .05$, all cases). Increases of PV and BV were more pronounced after both 3-week training loads (TPost1 and TPost2). No significant effect between times was found for PV or BV ($p > .05$, both cases). RCV was mainly unaffected by exercise ($p > .05$). The reported changes in the measured variables were not corrected for changes in PV.

Correlation

We looked at correlations between hepcidin, Fe, Hb, and hsCRP. A positive correlation between Hb and Fe concentration was observed at TPost1 and had a correlation

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**Figure 2** — Hepcidin concentrations measured at baseline (BPre), during the first and second training loads (TPost1, TPost2), after both recovery phases (Recovery1, Recovery2) and poststudy (BPost), $M \pm SD$. †Significantly different from baseline (BPre), $p < .05$. ‡Significantly different from first progressive training load (TPost1), $p < .05$. 
Discussion

The main findings of the current study indicate that serum hepcidin and sTfR were affected after 8 weeks of endurance running in women. Serum hepcidin concentrations were reduced at TPost1 and BPost, and sTfR increased in Recovery2 and BPost compared with BPre. No positive relation was found with inflammation.

This is the first study of different training intensities and durations and number of sessions completed during long-term endurance exercise and serial assessments of markers of inflammation, hepcidin, and iron status in female runners. The improvement in VO2max and the Cooper test results at BPost compared with BPre suggests that the recovery processes during the experiment were sufficient, resulting in a positive training effect in both groups. We did not observe significant changes in hsCRP, as previously reported after 9 weeks of basic combat training (Karl et al., 2010), or that prolonged training caused a decrease in inflammatory markers to a concentration below off-season concentrations (Nicolas et al., 2002). This should also be considered in our study, in which we observed depletion of serum ferritin at Recovery 2 and BPost, even below 12 μg/L in 6 of the 18 (33.33%) subjects, without a decrease in Hb concentration <120 g/L. These subjects would have already met the iron-deficiency criteria for women (Custer, Finch, Sobel, & Zettner, 1995). Further analyses on a larger sample of iron-deficient athletes are needed to evaluate hepcidin-response variability with regard to serum ferritin concentrations. It is also necessary to investigate mechanisms in female subjects with different concentrations of ferritin, particularly in those with ferritin <30 μg/L.

In clinical practice, this is not an uncommon finding in women and affects 64% of athletes and 87% of nonathletes (Di Santolo, Stel, Banfi, Gonano, & Cauci, 2008). In contrast to urinary hepcidin, there is a lack of data about the timeline of postexercise response in serum hepcidin. In addition, serum hepcidin does not correlate with urinary hepcidin (Dallalio, Fleury, & Means, 2003; Hoppe et al., 2009; Troadec et al., 2009). Therefore, it is
possible that in our study we missed the peak at the 14- to
24-hr observational time point. We should also have con-
sidered that we only compared baseline concentrations
(before and poststudy), instead of better rested control
conditions over the same time.

In our study, we observed significant decreases in
RBC and Hb markers after both training loads (TPost1
and TPost2). The estimated changes in PV and BV tended
to increase (TPost1 and TPost2) in our female runners,
as expected after several days of aerobic exercise. The
changes in Hb, RBC, and PV could be accompanied by
dilutional pseudoanemia as reported in numerous inves-
tigations as a normal adaptive response after prolonged
endurance-exercise training (Schumacher, Schmid,
Grathwohl, et al., 2002; Schumacher, Schmid, Konig, &
Berg, 2002; Zoller & Vogel, 2004). However, at BPre,
Recovery1, Recovery2, and BPost, PV expansion played
little or no role on the results of this study, because testing
took place after at least 2 days of rest (Reinke et al., 2010;
Schumacher, Schmid, Konig, & Berg, 2002). Taking these
limitations into account, sTfR is more recommended as
a marker of iron deficiency in athletes, because it is sug-
gested that exercise has no effect on sTfR levels (Schu-
macher, Schmid, Grathwohl, et al., 2002; Malczewska,
Blach, & Stupnicki, 2000; Malczewska, Raczyńska, &
Stupnicki, 2000). More markers of iron status, such as
transferrin, red-cell distribution width, and reticulocytes,
should be used to differentiate the mechanism by which
training affects iron status.

Limitations

We recruited only a moderate number of subjects, and,
thus, we may not have been able to detect more subtle
differences in laboratory parameters. It was also difficult
to draw definite conclusion for between-groups compar-
sions because running experience and training intensity
(in terms of sessions and distance covered) may have an
impact on the amount of inflammation, hepcidin, and,
ultimately, iron status. A shortcoming of the current study
is that we did not collect data on dietary intake throughout
the study. To strengthen the study, we should also take into
account the women’s menstrual cycles. This, however, is
more representative of everyday training practice. Finally,
our results may not be applicable to men and should also
be confirmed in elite athletes.

Conclusions and Clinical
Implications

In this study 33% of our subjects had laboratory markers
suggestive for iron deficiency after the end of prolonged
training. The 1-week recovery period after the race was
insufficient to normalize iron stores. Iron deficiency is
known to occur in stages, so prolonged training is likely
to increase the risk of depleted iron stores. Although
we did not observe any changes in Fe or inflammatory
parameters, one should consider the effects of cumulative
hepcidin increases on iron stores. We are therefore pro-
ponents of comprehensive iron-status evaluation to detect
possible functional or absolute iron deficiency in women
initiating or practicing regular physical activity.

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